DNA amplification lab report

Name: _____

1. Paste an image of your agarose gel and label ALL the fragments in the molecular weight ladder and on the lanes with their approximate size and sample ID. Based on the results of your experiment, answer the following questions.

Does the size of the bands on your gel correspond to the amplicon sizes expected? How did you calculate the size of your expected PCR product?

Why would you only get a positive reaction (amplification) with the primer set that matches your DNA source?

What could be the cause of a false positive reaction, this is, amplification with a primer set specific for a specie different from your source?

2. A successful PCR needs a complete mastermix. If one of the reagents is missing, you will have suboptimal amplification or no amplification at all. What would be the specific reason why your PCR reaction did not work if any of the following circumstances arose, assuming that the rest of the mastermix components are present and the PCR program is optimal:

There are no primers in the reaction.

There are no dNTPs in the reaction.

There is no *Taq* polymerase in the reaction or is not active.

3. After you run the product of a PCR reaction on an agarose gel you observe a lane with multiple bands (when you should only have one). Provide a potential explanation and propose a solution to correct the problem.

4. While running a gel you get distracted and when you come back you find: 1) no gel in the electrophoresis chamber (it disappeared!), 2) no bands on the sample lanes, only on the ladder, and 3) no bands on any lane. The equipment is working correctly, and the mistake must have occurred during the gel preparation or running. Explain what happened on each case.

5. Explain how PCR is used in real-world situations like disease diagnostic, forensic analysis of genetic testing.

6. Estimate the fragment size of the amplicons resulting from using the following primers on different *E. coli* genes and provide the name and function of it.

A. dnaK

F 5'-TACGGTCTGGACAAAGGCAC-3'

R 5'-ACGCGGCTCTTTACCAAAGA-3'

B. rpoS

F 5'-AACTGTTATCGCAGGGAGCC-3'

R 5'-TGTCCAGCAACGCTTTTTCG-3'

C. gyrB

F ACCCGGACAAACTGCGTTAT

R ACAATCGGCTCGAACAGGTT

D. ompA

F CCATGAAAACCAACTGGGCG

R CCGGTTTCAGGGTTGCTTTG

7. Calculate the DNA and water volumes needed to prepare the following solutions.

Original DNA concentration (ng/uL)	**Needed DNA concentration and volume**	**DNA volume**	**Water volume**
150	100 uL @ 5 ng/uL		
85	150 uL @ 1 ng/uL		
50	50 uL @ 10 ng/uL		
76	120 uL @ 2 ng/uL		
320	500 uL @ 10 ng/uL		
265	100 uL @ 5 ng/uL		
20	150 uL @ 1 ng/uL		
1256	50 uL @ 10 ng/uL		
100	120 uL @ 2 ng/uL		
58	500 uL @ 10 ng/uL		